INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCHEMICAL ANALYSIS AND *INVITRO* ANTIOXIDANT POTENTIAL OF *ANNONA MURICATA* L.

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Article Received on: 26/12/18 Approved for publication: 22/02/19

DOI: 10.7897/2230-8407.1004131

ABSTRACT

The traditional medical methods, especially the use of medicinal plants still play a major role in the developing countries. The history of the use of herbal medicine may be as old as the history of mankind. Many of the herbs and spices used by humans as food which yields useful medicinal compounds. *Annona muricata* L. leaves are traditionally used to treat diabetes. People have been consuming raw leaves of *Annona muricata* L. to control blood glucose levels. The acetone, methanol, hot water and successive water leaf extracts of *Annona muricata* L. were subjected to preliminary phytochemical screening of various plant constituents. The antioxidant potential of the *Annona muricata* L. leaves was established by total flavonoid content, FRAP assay, ABTS assay, Metal chelating activity, Phosphomolybdenum assay, Assay of superoxide radical scavenging activity, Free radical scavenging activity on DPPH along with the determination of total phenolic and tannin contents in the *Annona muricata* L. leaves extracts. This study aim is to evaluate bioactive constituents and *invitro* antioxidant activity of acetone, methanol, hot water and successive water extracts of *Annona muricata* L. leaf. Preliminary analysis revealed that all the four extracts responded positively for alkaloid, flavonoid, carbohydrate, glycosides, saponins, tannin, phytosterol and phenolics. The present study explored that *Annona muricata* L. shows efficient antioxidant activity and could act as safe and cost-effective with potential biological applications.

KEYWORDS: Phytochemical, Antioxidant, FRAP, ABTS and DPPH

INTRODUCTION

Due to its therapeutic potential *Annona muricata* L. has been widely studied in the last decades which are a species of the *Annonaceae* family. Due to its bioactivity and toxicity this species has attracted the attention and also the medicinal uses of the *Annonaceae* family were reported long time ago. *A. muricata* has been used as insecticide and parasiticide which were indicated by ethnobotanical studies[1]. Fruit juice and infusions of leaves or branches have been used to treat fever, sedative, respiratory illness, malaria. The leaves, bark, fruit and seed of *A. muricata* have been subject of countless medicinal uses[2]. The most widely used preparation in traditional medicine is the decoction of bark, root, seed or leaf and applications are varied. Unripe fruit, seeds, leaves and roots are also used as biopesticides, bioinsecticides and topical insect repellents. The importance of this species in pest control was indicated in the edition of ''Pesticide action and alternatives for Latin America", which recommended the use of aqueous extract of *A. muricata* to control lepidopteran larvae, aphids and thrips among others.

MATERAILS AND METHODS

Collection, Identification and Authentication

The dried aerial leaves of *Annona muricata* was obtained from Sulagiri forest, Krishnagiri District, Tamilnadu. The collected leaf materials were cleaned shade dried and powdered for further extraction and analysis.

Preparation of Methanol Extract

The extract of leaves of *Annona muricata* was prepared by refluxion technique. 50 gm of dried leaves was added to 1 lit round bottom flask and extracted with different solvents (methanol, acetone, hot water and successive water) in batches. After completion of extraction, all the extracts were filtered and concentrated by using rotary vacuum evaporator. The extracts were dried in vacuum dryer and stored at room temperature until used.

Phytochemical Screening

The different leaf extracts of *Annona muricata* were subjected to phytochemical screening of various plant constituents. 10mg extract was diluted per 10ml of the respective solvent to make the sample for preliminary tests. Then the extracts were tested for various chemical constituents with the help of different chemical tests.

In Vitro antioxidant studies

Determination of total phenolic and tannin contents

The whole phenolic content was analysed based on the technique described already[3]. Aliquots of respective extracts were taken and the measurement is prepared equal to 1 ml with distilled water. In respective tube (0.5 ml) folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added

in sequence. The test tubes were positioned in dark for 40 min, Afterward vortexing the reaction mixture, and at 725 nm the absorbance was note down counter to the reagent blank. The examination was done in triplicate and the outcomes were expressed as the tannic acid equivalents (TAE). By means of the similar extract the tannins were assessed later treatment with polyvinyl polypyrrolidone (PVPP). 100 mg of PVPP was balanced in a 100 × 12 mm test tube and to this 1.0 ml of distilled water and then 1.0 ml of tannin comprising phenolic extract were added. The content was vortexed and placed in the test tube at 4 ºC for 4 h. Then the sample was centrifuged (3000 g for 10 min at room temperature) and the supernatant was collected. This supernatant contains only simple phenolics excluding tannins (the tannins would have been precipitated together with the PVPP). The phenolic content of the supernatant was determined, as observed above and expressed as the content of non-tannin phenolics on a arid matter from the above results, the tannin content of the sample was calculated as follows[4,9]:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%).

Estimation of total flavonoid content

The total flavonoid content of sample extracts was determined by the use of a slightly modified colorimetric method described previously[5]. With 2 ml of distilled water, 0.5 ml extract was mixed and 0.15 ml of a 5% $NaNO_2$ solution was added subsequently. 0.15 ml of a 10% AlCl 3 solution was added after 6 min and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume of 5 ml, and then the mixture is thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as gram of rutin equivalent (RE) per 100 gram of extract[9].

Ferric-reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of samples was estimated according to the procedure described previously[6]. FRAP reagent (900 µl) was freshly prepared and incubated at 37°C, then 90 µl of distilled water was mixed along with 30 µl of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l FeCl 3. $6H_2O$ and 25 ml of 0.3 mol/l acetate buffer (pH 3.6) as already described[2]. Towards the end of incubation the absorbance values were noted down instantly at 593 nm, by means of a spectrophotometer. Methanolic solutions of well-known Fe (II) concentration, ranging from 100 to 2000 µmol/l, ($FeSO_4 .7H_2O$) were used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC 1) was defined as the concentration of antioxidant having a ferric-TPTZ reducing capability equivalent to that of 1 mmol/l $FeSO_4 . 7H_2O$. EC 1 was evaluated as the concentration of antioxidant giving an absorbance growth in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution, determined using the corresponding regression equation[4].

Antioxidant activity by the ABTS •+ assay

The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method described previously[6]. ABTS •+ was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was µmol/g sample extracts on dry matter diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.700 ± 0.02. The stock solution of the sample extracts were diluted such that later introduction of 10 µl aliquots into the assay, they formed between 20% and 80% inhibition of the blank absorbance. Later the addition of 1 ml of diluted ABTS solution to 10 µl of sample or Trolox standards (final concentration 0–15 µM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity[4,9].

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method described previously[8]. An aliquot of 100 µl of sample solution (in 1 mM dimethyl sulphoxide) was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100 g extract[9].

Assay of superoxide radical scavenging activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin–light–NBT system[10]. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg NBT and 100µl sample solution. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination, the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept in the dark served as blanks. The percentage inhibition of superoxide anion generation was calculated as:

$$\% \text{ Inhibition} = [(A\ 0 - A\ 1) / A\ 0] \times 100$$

where A 0 is the absorbance of the control, and A 1 is the absorbance of the sample extract/standard[9].

Free radical scavenging activity on DPPH

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method described previously[11]. Sample extracts at various concentrations was taken and the volume was adjusted to 100 µl with methanol. 5 ml of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula[9]:

% DPPH radical scavenging activity = (Control OD –Sample OD / Control OD) X 100

Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method described previously[10]. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (0.2 M, pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81(mol/l) -1 /cm. Extracts (10 µl) were added to 3.4 ml of phosphate buffer together with hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (reaction mixture without extract) and A_1 is the absorbance of the extract/standard.

Hydroxyl radical scavenging activity

The scavenging activity of acetone, hot water, methanol, hexane and successive water of *A.muricata* on hydroxyl radical was measured according to the method described previously. Various concentrations of extracts were added with 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26 EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80–90 °C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5%w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity (HRSA) is calculated by the following formula:

$$\% HRSA = \text{from } [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard[9].

Assay of nitric oxide scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH 7.4) was mixed with 100 µl sample solution of various extracts and incubated at room temperature for 150 min. The same reaction mixture without the sample was used as the control After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm[11].

β-carotene/linoleic acid antioxidant activity

One millilitre of a β-carotene solution in chloroform (1 mg/10 ml) was pipetted in to a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45 °C for 4 min and, 50 ml of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 200 µl of the antioxidant (extracts, BHA or α-tocopherol) solution at 1 mg/ml concentration and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the absorbance was monitored at 15 min intervals until 180 min. All determinations were carried out in triplicate. The antioxidant activity of the sample extracts and standard was evaluated in terms of bleaching of β-carotene using the following formula:

$$AA = [1 - (A_0 - A_t)/(A_{10} - A_{1t})] \times 100$$

where A_0 and A_{10} are the absorbance measured at zero time of incubation for the test sample and control, respectively, and A_t and A_{1t} are the absorbances measured in the test sample and control, respectively, after incubation for 180 min[9].

RESULTS

Extraction of crude extracts

The yield of the methanol, successive water, acetone and water extract from the *A. muricata* were 31.5, 12.0, 11.0 and 15.0% respectively.

Preliminary analysis of phytochemicals in different leaf extracts of *A. muricata*

All the four extracts responded positively for alkaloid, flavonoid, carbohydrate, glycosides, saponins, tannin, phytosterol and phenolics.

***In Vitro* antioxidant assay**

Determination of total phenolic content in different extracts of Annona muricata

Among the different extracts analyzed, hot water extract has recorded the highest phenolic content with the value of 901.42mg GAE/1g extract.

Determination of total tannin content in different leaf extracts of *Annona muricata*

The acetone leaf extract of *A.muricata* was found to contain highest tannin content of 401.42 mg GAE/1g) at 10000µg concentration.

Determination of total flavonoids content in different leaf extracts of *Annona muricata*

Acetone extract was found to contain maximum total flavonoid content with 799.33 mg RE/1g extract.

Ferric reducing antioxidant power (FRAP) assay in different extracts of *Annona muricata*

During the Ferric reducing antioxidant power (FRAP) assay acetone extract showed higher FRAP activity of 105.48 mmol Fe (II) E / mg.

$ABTS^{\bullet+}$ scavenging activity in different leaf extract of *Annona muricata*

Among the different leaf extracts of *A.muricata* hotwater extract showed the highest scavenging activity of 2652.73 µM TEAC/g extract.

Table 1. Characterization of crude extracts of methanol, successive water, acetone and water extracts of the *A. muricate*

Samples used	Mass (%)	Appearance and consistency
Methanol	31.5	Brownish colour and Solid
Successive water	12.0	Brownish colour and Solid
Acetone	11.0	Brownish colour and Solid
Water	15.0	Brownish colour and Solid

Table 2. Phytochemical test of different leaf extracts of *A. muricate*

S. No	Plant constituent	Test/Reagents	Extracts			
			MeOH	Acetone	Successive Water	Water
1	Carbohydrates	Molish''s test	+	+	+	+
		Fehling's test	+	+	+	+
		Barfoed's test	+	+	+	+
2	Alkaloids	Dragendroff's	-	-	-	-
		Hager's	-	-	-	-
		Mayer's	-	-	-	-
		Wagner's	-	-	-	-
3	Saponins	Foam test	+	-	-	+
4	Flavonoids	Shinoda test	+	+	+	+
		Lead acetate test	+	+	+	+
5	Steroids	Salkovski	+	+	+	+
		Liebermann-burchard	+	+	+	+
6	Amino acids	Ninhydrin test	+	+	+	+
		Millon's test	+	+	+	+
7	Proteins	Millon's test	-	-	-	-
		Biuret test	-	-	-	-
8	Tannins and Phenolic	Ferric chloride test	+	-	+	-
		Lead acetate test	+	-	+	-
		Bromine water test	+	-	+	-
9	GLYCOSIDES:	General test	+	+	+	+
	a) Anthraquinone	Borntrager's test	+	+	+	+
		Modified borntrager's test	+	+	+	+
		Hydroxyl Anthraquinone	+	+	+	+
	b)Cardiac	Kedde's test	-	-	-	-
		Raymond's test	-	-	-	-
		Legal's test	-	-	-	-
		Baljet's test	-	-	-	-
	d) Coumarins		+	+	+	_
	e) Cyanogenetic	Sodium picrate test	-	-	-	-
	f) Saponin	Froth formation test	+	+	+	+

Table 3. Total phenolic and tannin contents in different extracts of *Annona muricate*

Concentration of sample (µg/ml)	Phenolic contents				Tannin contents			
	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol extract mg GAE/1g	Acetone extract mg GAE/1g	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol Extract mg GAE/1g	Acetone extract mg GAE/1g
1000	196.66	420.38	261.42	64.76	66.19	170.38	252.57	114.28
2000	430.95	579.23	582.38	128.57	101.42	196.53	267.14	172.85
3000	596.66	602.30	690.38	156.66	140.47	223.84	280.95	205.23
4000	658.57	651.21	699.23	324.28	169.04	231.15	286.53	264.76
5000	760.00	686.92	739.04	460.95	189.52	260.38	291.42	290.47
6000	784.28	698.07	765.71	611.42	227.61	266.92	300.38	330.95
7000	829.04	698.07	789.52	673.80	270.93	277.30	305.23	340.47
8000	840.95	698.84	825.23	653.33	301.90	276.92	316.19	375.23
9000	851.42	713.07	849.04	783.33	350.47	283.84	327.61	383.33
10000	901.42	736.53	850.47	841.42	382.38	292.30	340.00	401.42

GAE; Gallic acid equivalent. Values are means of three independent analysis ± SEM (n = 3)

Table 4. Total flavonoids content and FRAP assay in different extracts of *Annona muricate*

Concentration of sample (µg/ml)	Flavonoids contents				FRAP assay			
	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol extract mg GAE/1g	Acetone extract mg GAE/1g	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol Extract mg GAE/1g	Acetone extract mg GAE/1g
1000	46.66	40.66	106.66	109.33	46.56	35.00	53.46	86.98
2000	83.33	65.33	136.66	116.00	47.29	36.66	53.72	89.54
3000	86.00	72.00	264.00	253.33	52.41	37.19	65.64	90.27
4000	140.00	131.33	292.66	280.66	54.89	37.76	70.76	94.00
5000	145.33	135.33	382.66	360.00	58.04	38.85	76.97	96.19
6000	240.66	182.66	482.00	464.00	60.38	39.25	77.41	97.88
7000	236.00	213.33	580.00	537.33	60.89	39.16	78.14	99.85
8000	242.66	216.66	660.66	576.66	60.59	40.74	84.35	102.85
9000	276.00	231.33	715.33	755.33	62.57	42.19	86.76	104.45
10000	301.33	249.33	772.00	799.33	66.81	42.63	87.93	105.48

RE: Rutin equivalent. Values are means of three independent analysis ± SEM (n = 3)
BHT: 91.81±2.15; Rutin: 216.59±2.59. Fe (II) E: Fe (II) equivalent. Values are means of three independent analysis ± SEM (n = 3).

Table 5. ABTS•+ scavenging activity in different extracts of *Annona muricate*

Concentration of sample (µg/ml)	Hot water extract (µM TEAC/g extract	Successive water (µM TEAC/g extract	Methanol extract (µM TEAC/g extract	Acetone extract (µM TEAC/g extract
100	2652.73	2510.98	2510.98	2267.98

BHT: 12454.2 ± 126.7; Rutin: 11096.8 ± 162.1. TEAC: Trolox equivalent antioxidant capacity. Values are means of three independent analysis ± SEM (n = 3).

Table 6. Phosphomolybdenum activity and Superoxide radical scavenging activity in different extracts of *Annona muricate*

Concentration of sample (µg/ml)	Phosphomolybdenum activity				Superoxide radical scavenging activity			
	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol extract mg GAE/1g	Acetone extract mg GAE/1g	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol Extract mg GAE/1g	Acetone extract mg GAE/1g
1000	577.22	508.88	658.33	581.11	6.82	9.84	7.57	1.51
2000	955.55	526.66	878.88	643.88	7.57	25.00	17.42	21.96
3000	1017.22	765.00	941.66	663.88	30.03	54.54	40.15	134.84
4000	1017.77	946.11	976.66	701.11	46.96	64.39	40.15	142.42
5000	1027.77	958.88	978.88	722.22	93.93	76.51	59.84	225.00
6000	1031.11	996.11	1002.77	731.11	122.72	96.21	78.78	278.03
7000	1027.77	1010.55	1003.88	732.77	124.24	176.51	79.54	406.81
8000	1034.44	1011.11	1016.66	781.66	185.6	189.39	144.69	445.45
9000	1034.44	1030.00	1027.77	886.11	342.42	250.00	190.15	487.12
10000	1115.00	1046.66	1029.44	942.77	375.00	265.90	193.61	525.75

BHT: 90.37±1.15; Rutin: 61.18±1.90. AAE: Ascorbic acid equivalent. Values are means of three independent analysis ± SEM (n = 3). BHT: 42.18±1.41; Rutin: 39.65±2.08.

Table 7. DPPH scavenging activity and Hydrogen peroxide scavenging activity in different extracts of *Annona muricate*

Concentration of sample (µg/ml)	DPPH scavenging activity				Hydrogen peroxide scavenging activity			
	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol extract mg GAE/1g	Acetone extract mg GAE/1g	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol Extract mg GAE/1g	Acetone extract mg GAE/1g
1000	1.50	1.74	2.32	45.24	50.98	52.94	32.74	43.13
2000	11.02	7.65	6.03	48.49	50.98	50.98	37.25	45.09
3000	17.16	10.44	14.50	50.23	50.98	50.98	37.25	45.09
4000	26.68	27.26	24.36	52.23	52.94	54.90	50.98	45.09
5000	27.03	32.25	30.39	57.30	54.90	56.86	50.98	47.05
6000	34.33	37.81	37.81	63.38	54.90	54.90	50.98	49.01
7000	36.89	66.70	45.47	66.82	58.82	56.86	54.90	50.98
8000	44.08	67.63	51.74	72.73	58.82	58.82	54.94	52.94
9000	46.86	94.31	66.00	73.89	60.78	64.70	56.86	56.86
10000	65.54	98.60	67.05	77.95	64.70	64.70	58.82	56.86

BHT: IC50 45.56 µg/ml; Rutin: IC50 24.71 µg/ml. IC50 - Inhibitory concentration. Values are means of three independent analysis ± SEM (n = 3).

Table 7. Nitric oxide scavenging activity and β-carotene/linoleic acid antioxidant activity in different extracts of *Annona muricate*

Concentration of sample (µg/ml)	Nitric oxide scavenging activity				β-carotene/linoleic acid antioxidant activity			
	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol extract mg GAE/1g	Acetone extract mg GAE/1g	Hot water extract mg GAE/1g	Successive water mg GAE/1g	Methanol Extract mg GAE/1g	Acetone extract mg GAE/1g
1000	25.00	5.68	10.79	27.95	2.57	10.57	21.42	20.85
2000	51.13	14.77	15.68	31.35	5.71	24.85	36.57	27.71
3000	68.75	18.63	27.27	34.09	23.71	38.57	36.85	32.57
4000	76.36	23.86	27.84	54.31	54.00	46.57	52.28	44.57
5000	58.52	26.13	30.68	55.11	56.57	68.85	58.85	61.14
6000	36.36	28.86	31.81	60.56	57.14	69.71	62.85	63.42
7000	35.22	53.97	33.52	60.90	69.71	70.85	72.00	63.71
8000	47.72	53.97	34.09	61.93	71.71	72.57	77.14	64.00
9000	35.22	63.75	57.72	63.06	75.14	75.71	77.71	69.71
10000	72.15	72.95	60.00	68.29	79.42	83.14	84.40	69.71

Phosphomolybdenum assay in different leaf extracts of *Annona muricata*

Among the different extracts, hot water extract has recorded the highest total antioxidant capacity of 1115.00mg AAE/g of plant extract.

Superoxide radical scavenging activity in different leaf extracts of *Annona muricata*

Among the different extracts the highest superoxide radical scavenging activity was found in methanol extract with 79.54%.

Free radical scavenging activity using DPPH in different leaf extracts of *Annona muricata*

The free radical scavenging activity of different leaf extracts was determined, among which successive water extract recorded total free radical scavenging capacity with IC50 value of 98.60 µg/ml.

Hydrogen peroxide scavenging activity in different extracts of *Annona muricata*

Among the different extracts the highest hydrogen peroxide scavenging activity was found in hotwater and successive water extract with 64.70%.

Hydroxyl radical scavenging activity in different extracts of *Annona muricata*

Among the different extracts the highest hydroxyl radical scavenging activity was found in successive water with 79.03%.

Nitric oxide scavenging activity in different extracts of *Annona muricata*

Among the different extracts the highest nitric oxide scavenging activity was found in hot water with 72.15 %.

β-carotene/linoleic acid antioxidant activity in different extracts of *Annona muricata*

Among the different extracts the highest β-carotene/linoleic acid antioxidant activity was found in methonal extract with 84.40%.

DISCUSSION

In the present study different solvents acetone, hot water, Successive water and methanol for *Annona muricata* were prepared from leaves and they were subjected to phytochemical screening, and antioxidant studies. Phytochemical analysis in different solvent leaf extracts of *A.muricata* inferred it was a good source of multiple phytoconstituents. The preliminary phytochemical tests indicated the presence of alkaloids, glycosides, saponins, tannins, phytosterols, phenolics and carbohydrates in various extracts of leaves of *A.muricata*. In *A. muricata* all the above mentioned compounds were present high in methanol extract followed by acetone, successive water and hotwater. Total phenolics, tannins and flavonoid contents were analyzed in successively extracted leaf sample. *In vitro* antioxidant activity was evaluated by chemical assays[12], such as DPPH, ABTS, ferric-reducing antioxidant power (FRAP), phosphomolybdenum reduction assay, superoxide anion radical scavenging, hydroxyl radical scavenging ,hydrogen peroxide scavenging, nitric oxide scavenging[13] and β-carotene antioxidant activity[1,4,9].

The total phenolic content was found to be high in hot water extract of *A. muricata.* The total tannin content, total flavonoid and ferric reducing activity was found to be high in acetone extract of *A.muricata.* The ABTS scavenging activity and phosphomolybdenum activity was found to be high in hot water extract of *A. muricata.* The superoxide radical scavenging activity was found to be high in methanol extract of *A.muricata* and the free radical scavenging activity using DPPH was found to be high in successive water extract of *A.muricata.* Hydrogen peroxide scavenging activity was found to be high in hot water and successive water extract of *A.muricata.* Hydroxyl radical scavenging activity was found to be high in successive water extract whereas nitric oxide scavenging activity was found to be high in hot water extract of *A.muricata.* β-carotene/linoleic acid antioxidant activity was found to be high in methanol extract of *A.muricata.*

CONCLUSION

To treat diabetes *Annona muricata* L. leaves are traditionally used. Raw leaves of *Annona muricata* L have been consumed by people to control blood glucose levels. The test for phytochemical analysis of various leaf extracts of *Annona muricata* L. inferred that it was a good source of multiple phytoconstituents. All the extracts showed multiple phytoconstituents. The results of various antioxidant assays revealed that *Annona muricata* L. leaves possess significant antioxidant potential. The present study explored that *Annona muricata* L. could act as safe and cost-effective with potential biological applications.

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Cite this article as:

Ravi Kumar K *et al*. Phytochemical analysis and invitro antioxidant potential of *Annona muricata* L. Int. Res. J. Pharm. 2019;10(4):95-101 http://dx.doi.org/10.7897/2230-8407.1004131

Source of support: Nil, Conflict of interest: None Declared

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